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Short Communication

Recurrent Herpes Simplex Virus Type 2 Meningitis: A Case Report of Mollaret's Meningitis

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SUMMARY: It is well known that herpes simplex virus (HSV) type 2 produces acute meningitis, while HSV type 2 rarely causes recurrent meningitis (Mollaret's meningitis). We report the history of a 40-year-old patient with recurrent HSV type 2 meningitis (Mollaret's meningitis). The patient had seven episodes of meningeal symptoms within a 7-year period. In the seventh episode, HSV type 2 DNA was confirmed by nested polymerase chain reaction (PCR) with the cerebrospinal fluid (CSF). A real-time quantitative PCR study of the first CSF sample detected 2,000 copies of the HSV genome, which rapidly disappeared following treatment with acyclovir. The present case may be the first case of HSV type 2 Mollaret's meningitis to be documented in Japan. In our case, HSV serum antibody titers were at low levels during the whole course of the disease. The possible pathophysiology of this case is discussed.

In 1944, Mollaret (1) became the first to describe a syndrome of benign recurrent aseptic meningitis, associated with cerebrospinal fluid (CSF) pleocytosis and elevated protein. Each episode was followed by spontaneous complete recovery. This syndrome has been described as being associated with intracranial tumors, collagen diseases, and various viral infections, including herpes simplex virus (HSV) types 1 and 2, Epstein-Barr virus (EBV), and other infections (2). Yamamoto et al. were the first to report a case of Mollaret's meningitis with polymerase chain reaction (PCR) confirmation of HSV type 1 DNA (3). Since then, HSV DNA, mostly HSV type 2, has also been detected by the CSF PCR amplification technique in patients with HSV Mollaret's meningitis (4-12).

We report the history of a patient with HSV Mollaret's meningitis, in whose CSF the HSV type 2 genome was detected by nested PCR; samples were further analyzed by the recently developed technique of real-time PCR.

In the first episode that took place in August of 1993, a 33-year-old man noticed a few vesicles on his penis; severe pain followed in the bilateral inguinal regions. The patient was diagnosed by a urologist as having genital herpes, and was treated with vidarabine salve. One week following the initial presentation, he developed a severe headache, fever, nausea, neck stiffness, and back pain, and was admitted to the hospital. The patient revealed only signs of meningeal irritation. A CSF examination showed marked pleocytosis. The patient was immediately treated with intravenous acyclovir (10 mg/kg three times a day) for 7 days; all symptoms resolved within the first 5 days of this therapy. He subsequently had six similar episodes of recurrent meningitis within a 7-year period (Fig. 1). In the third and fourth attacks, genital herpes preceded or was associated with recurrent meningitis. In the second attack and after the fifth attack, the patient did not notice genital herpes. At the second episode, the HSV genome was detected in the CSF by a single PCR; the type of HSV was not examined at that time. The patient was readmitted to our hospital during the sixth and seventh episodes.

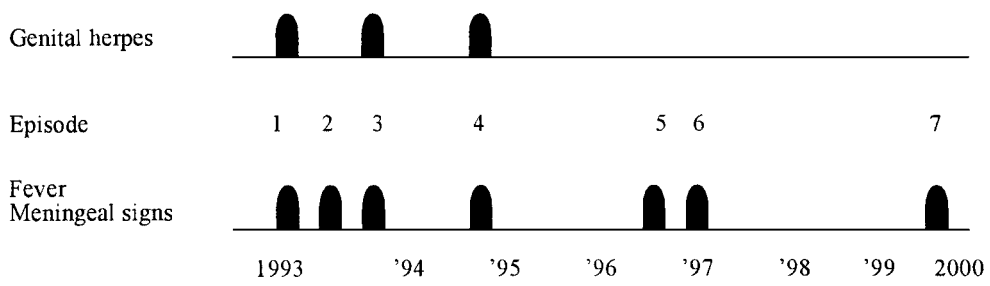

Fig. 1. Clinical course.

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On his most recent admission of October 2000, the patient, 40 years old, showed nuchal rigidity and positive Kernig's sign, similar to the symptoms expressed during his previous illnesses. Results of studies for collagen diseases were negative. His CD4/CD8 ratio was normal. The serum IgE level was elevated at two times the normal range, whereas serum IgG, IgA, IgM, and complement levels were within the normal range. A CSF examination showed mononuclear pleocytosis (736/mm^3, 87% lymphocytes and monocytes, and 13% neutrophils without Mollaret's cells) and an increased protein level (184 mg/dl), but a normal glucose level (51 mg/dl) was observed. A virus isolation test using VERO cells was negative.

His serology tests and PCR results during the entire course of the disease are shown in Table 1. From the acute to convalescent stages of the most recent episode, the serum HSV complement fixation (CF) titer was 1:4; HSV type 1 and HSV type 2 neutralizing antibodies (NT) using the microplate method were 1:8 and 1:4 (positive≧1:4), respectively; HSV enzyme-linked immunosorbent assay (ELISA) IgG was 11.4 (positive≧4). For CF and ELISA, HSV type 1 antigen (HF strain) was used. Serologic tests for mumps, measles, rubella, varicella-zoster, influenza, enterovirus, EBV, and mycoplasma were all negative. An IgG ELISA kit using HSV type-specific glycoprotein G (gG) was used for the differentiation of HSV types 1 and 2 (13). HSV gG-1 was found to be 0.082 (positive≧1), and HSV gG-2 was 3.482 (positive ≧ 1).

Nested PCR and real-time PCR were performed for serum and CSF taken during the acute stage and following recovery. For nested PCR, the DNA was prepared from CSF and serum using a DNA Extractor Kit (WAKO Pure Chemical Industries, Ltd., Osaka). The conserved HSV DNA polymerase region was amplified by the first round of PCR using the primer pair described by Rozenberg and Lebon (14). The 512 bp fragment was confirmed by Southern blot hybridization with an HSV-specific probe. The primers for nested PCR were 5′-GACTACCTGGAGAGATCGAGGT-3′ and 5′-CGAGTTACACACGACATTGA-3′, and the PCR product was 198 bp. When HSV type 1 or HSV type 2 DNA was present in the mixture, a 198-bp sequence was amplified by nested PCR. Within this sequence, individual virus strains have unique restriction sites, such that the amplification product can be typed by digestion with *Sma*I and *Bam*HI.

The HSV-specific 198-bp DNA band was amplified by nested PCR from the initial CSF, but not from the serum. The PCR product was further digested with the restriction enzyme *Bam*HI, resulting in the identification of HSV type 2.

For real-time PCR, the DNA absorbed to the QIAamp spin column was eluted with 50 μl of distilled water and then was subjected to PCR. The sequences of the PCR primers and that of the probe were selected from the major capsid protein region of HSV. The forward and reverse primers were 5′-TGTTTTACCTGCTGCARGCC-3′ and 5′-TAGGTCACG AYGTACGAGAGACCA-3′, respectively. The TaqMan probe selected between both primers was fluorescence labeled with 6-carboxy-fluorescein at the 5′ end as the reporter dye, and 6-carboxytetramethylrhodamine at the 3′ end as the quencher (5′-HEX-AGTGCATCACCAGCTACTGGAACAACACGC-TAMRA-3′). The primers and the TaqMan probe were purchased from Greiner Japan, Tokyo. The PCR product was detected by increases in fluorescence using ABI PRISM 7700 (PE Biosystems, Foster City, Calif., USA).

Two thousand copies/ml of the HSV genome were quantified in the initial CSF, with none in the serum. Real-time PCR of the CSF sample taken 14 days later showed that the HSV genome had disappeared during treatment.

As before, the patient responded well to intravenous acyclovir treatment; all of his symptoms resolved within 1 week. He was then given oral valacyclovir with the goal of preventing recurrent HSV meningitis; however, the valacyclovir was discontinued due to the side effect of abdominal pain.

Table 1. Serologic antibody testing and PCR study results

Episode	CSF cell (/mm^3)	CSF protein (mg/dl)	Serum titer	CSF titer	CSF PCR	Acyclovir therapy
1	1147	232	HSV-1 (NT) <4→4 HSV-2 (NT) <4→<4	HSV(CF) <1		1500 mg×7 days
2	84	37	HSV-2 (NT) <4	HSV IgG (ELISA) 2.2 HSV IgM (ELISA) 1.6	HSV (+)	1500 mg×14 days
3	49	55	HSV-2 (NT) <4	HSV-1,2 (NT) <1		1000 mg×7 days
4	229	48	HSV (CF) <4	HSV-1,2 (NT) <1		1000 mg×10 days
5	457	118	HSV (CF) <4	HSV-1,2 (NT) <1	(–)	750 mg×7 days
6	125	55	HSV (CF) <4	HSV (CF) <1	(–)	750 mg×5 days
7	736	184	HSV (CF) 4→4 HSV-1 (NT) 8→8 HSV-2 (NT) 4→4 HSV IgG (ELISA) 11.4→9.4 Serum HSV PCR* (–)	HSV (CF) <1 HSV-2 (NT) <1 HSV IgG (ELISA) 0.2	HSV-2** (+) Real-time HSV PCR 2,000 copies/ml	750 mg×14 days

CSF, cerebrospinal fluid; HSV-1, herpes simplex virus type 1; HSV-2, type 2; CF, complement fixation test; NT, neutralizing test; ELISA, enzyme-linked immunosorbent assay.
*nested PCR and real time PCR.
**nested PCR

Mollaret's meningitis is defined as a benign recurrent aseptic meningitis characterized by three to ten episodes of fever and signs of meningeal irritation lasting between 2 and 5 days, associated with spontaneous recovery (1,2). Yamamoto et al. first reported a case of Mollaret's meningitis in the United States, with PCR confirmation of HSV type 1 DNA (3). To date, approximately 50 cases of recurrent HSV meningitis have been described in the United States and in Europe (4-12); HSV type 2 has been responsible for the majority of these cases, with the exception of a few cases of HSV type 1, EBV, etc. These findings are compatible with the fact that HSV type 2 more frequently causes acute meningitis than does HSV type 1 (15). However, in Japan, the present case appears to be the first documented case of HSV type 2 Mollaret's meningitis; this case may be attributed to inattention to the fact that HSV type 2 can produce Mollaret's meningitis. When patients with recurrent viral meningitis are encountered, PCR analysis with CSF for HSV and type-specific ELISA testing should be conducted.

With regard to the reported cases of HSV type 2 Mollaret's meningitis, women are typically more frequently affected than men. The interval between episodes ranges from weeks to years (on an average of several years), and three to ten attacks may occur. After the earliest attacks, recurrent meningitis often lacks an association with genital herpes, as in our case. However, the possibility that genital lesions might simply be asymptomatic should be noted. In Japan, HSV type 1 rather than HSV type 2 is the primary causes of genital herpes (16); however, recurrent cases of genital herpes are primarily caused by HSV type 2, as is the case in other countries.

Some question has arisen as regards the pathogenesis of HSV type 2 Mollaret's meningitis. For example, it remains unknown how HSV type 2 reaches the central nervous system from the primary infection site. Proposed hypotheses suggest either possible neural or hematogenous routes. HSV type 2 usually stays in the latent phase or at a low level of infectivity in the lumbo-sacral sensory ganglia. Once it is reactivated, it can cause recurrent muco-cutaneous illness through peripheral nerve spread from the ganglia. Venot et al. (11) demonstrated that the same HSV type 2 strain caused meningitis in a patient with recurrent genital herpes by using PCR analysis together with a restriction enzyme technique. In our case, the HSV genome was detected in the CSF, but not in the blood samples, as shown by various PCR studies. A previous report also suggested that HSV type 2 infection does not originate from hematogenous spread, as blood samples were negative for HSV PCR (6). This evidence further supports the hypothesis of centripetal spread from the peripheral reactivation in the sensory ganglia to the meninges. In addition, frequently recurring meningitis with or without genital herpes is probably due to the particular features of HSV type 2, which frequently causes latent infection in the sensoryganglia of the lumbo-sacral spinal cord.

It should be noted that in all episodes of our case, HSV serum antibody titers by CF, NT, or previous ELISA were at low levels, whereas blood immunoglobulin values were normal. Many reported cases of recurrent HSV type 2 meningitis have been associated with positive antibody testing. However, a few of the reported cases probably also showed a weak response; for example, Cohen et al. (8) have described a case that was seronegative, and Picard et al. (6) have described one case that was positive for both HSV type 1 and type 2. Nakata et al. (unpublished data, 2000) reported idiopathic CD4 T-lymphocytopenia in a patient with HSV Mollaret's meningitis. In our case, the low NT response, including previous ELISA titers, may have been involved in the pathogenesis of the recurrence. Use of the HSV type 1 antigen upon CF or ELISA may be related to a low HSV type 2 antibody level. However, it may also be the case that rapid acyclovir therapy suppresses antibody production. Further studies will be needed in order to examine these possibilities.

Prevention therapy may be necessary in cases of frequent recurrent meningitis, as well as in cases of recurrent genital herpes (17). We initiated oral valacyclovir therapy to prevent recurrence, but treatment was stopped due to the side effects. When patients present with HSV Mollaret's meningitis, long-term suppressive therapy or patient-initiated therapy should nonetheless be considered.

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